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Two-Stage and Weibull Models for Carcinogenesis Applied to the ED_{01} Discontinued Dosing Data

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The two-stage clonal expansion model for a single, less-than-lifetime period of dosing is formulated and applied to the liver and bladder tumor data from the ED_{01} study. The model successfully predicts liver tumor incidence for time points beyond termination of dosing with 2-acetylaminofluorene, but it is unsuccessful for bladder tumor incidence. A discontinued dosing version of the Weibull model is proposed and is shown to predict successfully both liver and bladder tumor incidences for time points after termination of dosing.

Introduction

The ED_{01} study (*1*) was conducted at the National Center for Toxicological Research (NCTR) for the purpose of establishing the shape of the tumorigenic dose-response curve for 2-acetylaminofluorene (2-AAF) at low doses (i.e., doses in the neighborhood of a 1% response rate). It was found that for the two primary carcinogenic responses, hepatocellular neoplasms of the liver and carcinomas of the bladder, distinctly different dose-response relationships occurred under a continuous dosing regimen. Whereas the tumorigenic dose response in the liver was nearly linear, that in the bladder was decidedly nonlinear. These contrasting shapes were observed at various points during the lifetimes of the BALB/c female mice that were used as test animals (*1*).

One aspect of the ED_{01} study that, in addition to its size, sets it apart from most other carcinogenesis bioassays is its inclusion of groups of animals for which dosing with the test chemical was discontinued several months prior to sacrifice. These groups are useful for advancing theories about the carcinogenic process. Indeed, Littlefield et al. (*2*) postulated the precursor role of "persistent" hyperplasia in 2-AAF-induced bladder cancer using information from the discontinued dosing groups. Day and Brown (*3*), working within the theoretical framework of the multistage model (*4*), observed that the discontinued dosing responses were consistent with the characterization of 2-AAF as an early-stage carcinogen in the liver and a late-stage carcinogen in the bladder.

Formal mathematical modeling of the ED_{01} study's discontinued dosing data was first done by Brown and Hoel (*5*). They derived a mathematical expression for the multistage model under discontinued dosing and fit it to the liver tumor prevalence data observed in sacrificed animals. They did not attempt to model the bladder tumor data, due to low responses in many treatment groups. Brown and Hoel (*5*) found the liver tumor prevalence data to be consistent with both a multistage model with four total stages, two stages being dose related, and a multistage model with six total stages, only one stage being dose related. Freedman and Navidi (*6*) included the ED_{01} data among various data sets that they examined in the context of the multistage model. Whereas their data selection and assumptions were slightly different from those of Brown and Hoel (*5*), Freedman and Navidi (*6*) found a multistage model with seven total stages, two being dose related, to give the best prediction of liver tumor response rates in the discontinued treatment groups. They were unable to find a version of the multistage model that would fit a selected portion of the continuous dosing bladder tumor data and successfully predict bladder tumor responses in the discontinued dosing groups. Chiang and Conforti

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(*7*) postulated a new model for time to onset of the tumorigenic response, including a version to describe discontinued dosing data. Working with only the ED_{01} bladder tumor data, they were unable to predict consistently the observed responses in the discontinued dosing groups.

Although previous investigators have been able to describe successfully the ED_{01} discontinued dosing liver tumor responses within the framework of the multistage model, none has yet modeled successfully the ED_{01} bladder tumor data for discontinued dosing. Indeed, it has been shown that acceptable model fitting for continuous dosing data cannot be taken to imply correctness of the model for extrapolating to discontinued dosing situations (*6,7*). The purpose of this paper is to investigate the currently popular two-stage clonal expansion model (*8,9*) with respect to its ability to describe the ED_{01} discontinued dosing data. The success of such modeling would perhaps enable the characterization of 2-AAF-induced liver and bladder tumorigenesis in terms of initiation, promotion, and completion (progression) (*10*). At any rate, successful prediction of bladder tumor responses for discontinued dosing groups by the two-stage clonal expansion model would add to its growing acceptance for carcinogenesis dose-response modeling.

Two-Stage Clonal Expansion Model

In recent years there has been movement away from the multistage model toward a model with only two distinct stages (e.g., two mutations or other genetic events) but with proliferation of cells that have undergone the first stage. One popular formulation of the model was proposed by Moolgavkar and his co-workers (*8,11*). Another widely recognized version of the two-stage clonal expansion model has been developed and applied by Greenfield et al. (*9*). As pointed out by Bogan (*12*), the original two-stage model formulated by Armitage and Doll (*13*) provided for cell proliferation. However, the most widely studied and applied version of the multistage model does not (*4*). Whereas the growth of cells that have undergone the first stage in the two-stage clonal expansion model is exponential, Bogen (*12*) argued that geometric growth appeared to provide a better representation of certain data sets. In this paper; the two-stage model with exponential cell growth will be studied, with the terminology of Thorslund et al. (*10*) being used to characterize carcinogenic action. Specifically, carcinogenic action will be modeled in terms of initiation, promotion, and completion (progression), where initiation and completion refer to the occurrence of the first- and second-stage events, respectively, in the two-stage model, and promotion refers to the growth of initiated cells.

For continuous exposure to a carcinogenic agent at a dose rate d between time 0 and time t, the instantaneous hazard (or age-specific incidence) rate of the two-stage model with exponential growth of initiated cells may be written as

$$h(d,t) = \int_0^t \lambda_1(d,u)\,\lambda_2(d,t)\,N(u) \exp\left\{\int_u^t \delta(d,v)dv\right\} du, \quad 0<t,$$

where λ_1 and λ_2 represent, respectively, the rates of occurrence of the first- and second-stage genetic events, δ represents the net birth minus death rate of first-stage cells, and N is the number of normal cells at risk of a first-stage event. The cumulative hazard function is

$$H(d,t) = \int_0^t h(d,u)\,du, \quad 0<t.$$

Whereas the genetic event rates and the cell growth rates may conceivably take various functional forms, the usual assumption is made here that they are independent of time and linear in dose. Thus $\lambda_1(d,t) = \alpha_1+\beta_1 d$, $\lambda_2(d,t) = \alpha_2+\beta_2 d$ and $\delta(d,t) = \alpha_3+\beta_3 d$. Furthermore, it is assumed that the number of normal cells at risk is so large that it may reasonably be regarded as constant. Under continuous dosing, then, the cumulative hazard function is

$$H(d,t) = N\frac{(\alpha_1+\beta_1 d)(\alpha_2+\beta_2 d)}{(\alpha_3+\beta_3 d)^2}\left\{\exp[(\alpha_3+\beta_3 d)t] - 1 - (\alpha_3+\beta_3 d)t\right\}, \quad 0<t.$$

For a single period of dosing at a constant rate d from time 0 to time t_1, followed by a period of discontinued dosing up to time t, the cumulative hazard for the two-stage clonal expansion model under the above formulation is (see appendix)

$$H(d,t;0,t_1) = N\frac{(\alpha_1+\beta_1 d)(\alpha_2+\beta_2 d)}{(\alpha_3+\beta_3 d)^2}\left\{\exp[(\alpha_3+\beta_3 d)t_1] - 1 - (\alpha_3+\beta_3 d)t_1\right\} + N\frac{(\alpha_1+\beta_1 d)\,\alpha_2}{(\alpha_3+\beta_3 d)\,\alpha_3}\left\{\exp[(\alpha_3+\beta_3 d)t_1] - 1\right\}\left\{\exp[\alpha_3(t-t_1)] - 1\right\} + N\frac{\alpha_1\,\alpha_2}{\alpha_3^2}\left\{\exp[\alpha_3(t-t_1)] - 1 - \alpha_3(t-t_1)\right\}, \quad t_1 \le t.$$

With this notation, the cumulative hazard under continuous dosing would be designated by H(d,t;0,t). Vari-

ous special cases of the two-stage clonal expansion model may be obtained by setting selected coefficients of $d(\beta_i)$ equal to zero in the continuous and discontinuous versions of the cumulative hazard function. The pure initiator model arises when $\beta_2 = \beta_3 = 0$, the pure completer model when $\beta_1 = \beta_3 = 0$, and the pure promoter model when $\beta_1 = \beta_2 = 0$ (*14*). The initiator-completer model arises when $\beta_3 = 0$, the initiator-promoter model when $\beta_2 = 0$, and the promoter-completer model when $\beta_1 = 0$.

Due to the fact that the rates of occurrence of the two genetic events appear as a product in the two-stage clonal expansion model, they are not, in general, separately identifiable. For continuous dosing data only, the models for pure initiator and pure completer are indistinguishable, although they can be distinguished with discontinued dosing data (*14*). The same is true for the initiator-promoter and promoter-completer models. Thus, if discontinued dosing data are available, then the pure initiator, pure completer, initiator-promoter, and promoter-completer models can all be resolved in terms of parameter estimation and model fit. However, if parameters are estimated and goodness-of-fit is ascertained using continuous data only, then extrapolation to situations of discontinued dosing, although it may be carried out, is very dependent on which of the indistinguishable continuous-dosing models is assumed. A different extrapolation problem arises for the initiator-completer model and for the full initiator-promoter-completer model. Although these models are identifiable for both continuous dosing and discontinued dosing data, the continuous dosing version of neither model can be extrapolated to a discontinued dosing situation, since individual parameters in the genetic event rates are nonidentifiable. Only the pure promoter model is uniquely identifiable irrespective of the dosing pattern.

ED_{01} Liver and Bladder Tumor Data

The ED_{01} study (*1*) was conducted in six animal rooms. Each was set up as a replicate of the experiment, to be loaded sequentially as animals became available from the NCTR breeding colony. For the various continuous dose groups (0, 30, 35, 45, 60, 75, 100, and 150 ppm 2-AAF), interim sacrifices were scheduled at 9, 12, 14, 15, 16, 17, and 18 months on study, with the earliest sacrifices being omitted in the lowest dose groups. Due to higher than expected survival and lower than expected bladder tumor rates by the time of the 18-month sacrifice in the first three rooms, some of the later scheduled sacrifices (including 18 months) were rescheduled for 24 months in the last three rooms. Thus, the ED_{01} study's 18-month sacrifice data came from the first three rooms only, while its 24-month sacrifice data came from the last three rooms only.

For the four highest dose levels of 2-AAF, discontinued dosing was included in the study's design. For each of these dose levels, groups of animals received 2-AAF for either 9, 12, or 15 months after which dosing was discontinued until sacrifice. Sacrifices were all originally scheduled for 18 months, but as indicated above, those in the last three rooms were rescheduled for 24 months.

Previous modelers of the ED_{01} tumor data have used various versions of the observed data for modeling purposes. Brown and Hoel (*5*) used only the liver tumor prevalence data observed in sacrificed animals, implicitly assuming that liver tumors were not related to the deaths of animals that did not survive to scheduled sacrifice. Chiang and Conforti (*7*) used bladder tumor rates observed in combined sacrificed and dead/moribund animals, also implicitly assuming no relationship between bladder tumors and the deaths of the animals. Freedman and Navidi (*6*) used bladder and liver tumor prevalence data from sacrificed animals as if the data were incidence data, i.e., they assumed that tumors observed at a sacrifice arose during the month of sacrifice. Since the two-stage model with clonal expansion is, as are all other models of tumorigenesis, a model for the distribution of time to onset of tumors, it is important that the tumor data used for model fitting represent as closely as possible the observed distribution of time to tumor onset.

In this paper, the nonparametric estimator of Kodell et al. (*15*) is used to adjust the observed tumor data with respect to censoring by competing risks. Similar adjustments have been proposed by Dinse and Lagakos (*16*) and Turnbull and Mitchell (*17*). With these methods, each animal's cause of death needs to have been determined by the examining pathologist. One of the strengths of the ED_{01} pathology data is that cause of death was reported for each animal that was removed as dead or moribund from the experiment. Hence, an adjustment for death from competing risks could be made. With the method of Kodell et al. (*15*), the distribution of time to tumor onset is estimated nonparametrically for each dose group separately. Thus it is possible to obtain dose-response information at any point in time by using the value of the time to tumor distribution function from each dose group at that time. In the present study, the time points of primary interest were 18 and 24 months, the sacrifice times in the discontinued dosing groups. Both hepatocellular adenomas and carcinomas were included as liver tumors, while only carcinomas were included as bladder tumors.

The data used for model fitting are given in Tables 1 and 2 for liver and bladder tumors, respectively. $\hat{P}$ represents the nonparametric estimate of the probability of tumor onset by time T (18 or 24 months), X is the number of animals observed with tumors up to time T, and $\hat{N} = X/\hat{P}$ is the "effective" sample size. That is, for given $\hat{P}$, $\hat{N}$ is the number of animals that would have been put on test initially, if time to tumor onset of each animal could have been observed, and if exactly X animals of the $\hat{N}$ would have developed tumors by time T. Data on all dead, moribund, and sacrificed animals are reflected in the numbers in Tables 1 and 2, even though only times corresponding to sacrifices at 18 and 24 months have been selected for evaluation.

Due to the nesting of the 18- and 24-month sacrifices

Table 1. ED_{01} liver tumor data adjusted for competing risks.

	Rooms 141, 142, 143 combined[a]					Rooms 144, 145, 146 combined[a]				
D[b]	T_1[b]	T[b]	$\hat{N}$[c]	X[c]	$\hat{P}$[c]	T_1	T	$\hat{N}$	X	$\hat{P}$
0	24	24	525	18	0.034	18	18	435	2	0.005
30	24	24	1432	114	0.080	18	18	1329	21	0.016
35	24	24	996	97	0.097	18	18	667	10	0.015
45	24	24	555	82	0.148	18	18	708	17	0.024
60	24	24	565	101	0.179	18	18	654	18	0.028
75	24	24	481	110	0.229	18	18	565	17	0.030
100	24	24	253	77	0.304	18	18	274	15	0.055
150	24	24	261	129	0.494	18	18	190	13	0.068
60	9	24	137	17	0.124	9	18	200	1	0.005
60	12	24	154	18	0.117	12	18	200	2	0.010
60	15	24	160	25	0.156	15	18	199	5	0.025
75	9	24	85	16	0.188	9	18	131	5	0.038
75	12	24	100	21	0.210	12	18	132	6	0.045
75	15	24	113	22	0.195	15	18	130	5	0.038
100	9	24	38	6	0.158	9	18	64	1	0.016
100	12	24	47	10	0.213	12	18	65	0	0.000
100	15	24	49	12	0.245	15	18	64	1	0.016
150	9	24	51	15	0.294	9	18	77	6	0.078
150	12	24	48	16	0.333	12	18	64	3	0.047
150	15	24	38	10	0.256	15	18	70	5	0.071

[a]A sacrifice at 18 months occurred only in rooms 144, 145, 146; a sacrifice at 24 months occurred only in rooms 141, 142, 143.

[b]D represents dose of 2-AAF in parts per million; T_1 represents time of termination of dosing expressed in months; T represents time of observation of time-to-tumor onset distribution expressed in months.

[c]$\hat{P}$ is the nonparametric estimate of the probability of tumor onset by time T; X is the actual number of tumors observed up to time T; $\hat{N}=X/\hat{P}$ is the "effective" sample size.

within rooms (and, consequently, much of the data on dead/moribund animals), it was decided to estimate time to tumor distributions using combinations of three animal rooms, grouped according to common sacrifice time. Although this approach confounds room differences with evaluation time differences, it does provide a starting

Table 2. ED_{01} bladder tumor data adjusted for competing risks.

	Rooms 141, 142, 143 combined[a]					Rooms 144, 145, 146 combined[a]				
D[b]	T_1[b]	T[b]	$\hat{N}$[c]	X[c]	$\hat{P}$[c]	T_1	T	$\hat{N}$	X	$\hat{P}$
0	24	24	1176	8	0.007	18	18	1111	2	0.002
30	24	24	2179	17	0.008	18	18	1613	5	0.003
35	24	24	1628	7	0.004	18	18	1176	2	0.002
45	24	24	1043	12	0.012	18	18	952	6	0.006
60	24	24	652	6	0.009	18	18	294	3	0.010
75	24	24	699	16	0.023	18	18	488	2	0.004
100	24	24	220	38	0.173	18	18	255	13	0.051
150	24	24	329	264	0.802	18	18	337	179	0.531
60	9	24	152	3	0.020	9	18	184	0	0.000
60	12	24	198	4	0.020	12	18	190	0	0.000
60	15	24	116	0	0.000	15	18	196	1	0.005
75	9	24	78	1	0.013	9	18	128	2	0.016
75	12	24	130	2	0.015	12	18	132	0	0.000
75	15	24	106	2	0.019	15	18	131	1	0.008
100	9	24	35	0	0.000	9	18	64	1	0.016
100	12	24	43	2	0.047	12	18	67	2	0.030
100	15	24	46	2	0.043	15	18	64	0	0.000
150	9	24	48	9	0.188	9	18	63	4	0.063
150	12	24	52	18	0.346	12	18	64	15	0.234
150	15	24	49	21	0.429	15	18	68	25	0.368

[a]A sacrifice at 18 months occurred only in rooms 144, 145, 146; a sacrifice at 24 months occurred only in rooms 141, 142, 143.

[b]D represents dose of 2-AAF in parts per million; T_1 represents time of termination of dosing expressed in months; T represents time of observation of time-tumor-onset distribution expressed in months.

[c]$\hat{P}$ is the nonparametric estimate of the probability of tumor onset by time T; X is the actual number of tumors observed up to time T; $\hat{N}=X/\hat{P}$ is the "effective" sample size.

point for investigating potential lack of fit of models. That is, if prediction of tumor rates for discontinued dosing groups based on models fit to continuous dosing data should break down due to room differences, it would still be possible to perform such predictions individually for 18 and 24 months (i.e., within groups of rooms). Whereas using tumor data from all six rooms taken together overcomes the confounding just mentioned, any model breakdown that might be due to room differences could not be so identified. Certainly for collapsed data, tumor probability estimates at 18 months are heavily influenced by the first three rooms and those at 24 months by the last three rooms.

Model Fitting

The nonlinear regression procedure (NLIN) in SAS (*18*) was used for fitting various versions of the two-stage model with cell proliferation to the tumor data in Tables 1 and 2. Weighted least squares was employed, with reciprocals of estimated variances being used for weights. The weighted sum of squares that was minimized is

$$\sum_{i=1}^{m} \frac{N_i(P_i - \hat{P}_i)^2}{(\hat{P}_i + \frac{1}{\hat{N}_i})(1 - \hat{P}_i + \frac{1}{\hat{N}_i})}$$

where $\hat{P}_i$ and $\hat{N}_i$ are the values of the nonparametric estimate of the probability of tumor onset by time T, and the "effective" sample size, respectively, from Table 1 or Table 2 for the *ith* group; $1/\hat{N}_i$ is a correction for continuity; P_i is the predicted probability of tumor, 1-exp {-H(D,T;0,T_i)}, from the two-stage clonal expansion model for the *ith* group; and m is the number of distinct groups (distinct combinations of D, T_l and T in Table 1 or Table 2) used for fitting the model. Since the sum of squares minimized is asymptotically a chi-square random variable, the estimation method is a modified minimum chi-square method.

Computationally, the Gauss-Newton method, the Marquardt method, and the derivative-free multivariate secant method (designated as DUD in NLIN) were used to determine the direction and distance for each succeeding iteration. All parameters were constrained to be nonnegative using BOUNDS statements.

To assess goodness-of-fit, a chi-square statistic like the one minimized was used, except that predicted values of P_i obtained from the model, rather than observed values, $\hat{P}_i$, were used to calculate variances in the denominator. If the fit of the model to a set of data points used to estimate the model's parameters was being assessed, then the degrees of freedom for chi-square were calculated as the difference between the number of data points and the number of estimated parameters. If the fit of the model to a separate set of data points not used for fitting the model was being assessed, then the degrees of freedom for chi-square were taken was the number of data points.

Results for Two-Stage Clonal Expansion Model

For each of the two tumor types, the first step was to fit various versions of the model to all 40 data points in order to ascertain which versions should be eliminated and which warranted further investigation. Those submodels of the general model that fit acceptably to all the data points were further studied by fitting them to only the 16 continuous dosing data points and then assessing their predictive ability for the 24 discontinued dosing data points, where possible.

For the liver tumor data (Table 1), the full, six-parameter model fit acceptably to all 40 data points ($p = 0.76$), as did the four-parameter initiator-promoter model ($p = 0.40$) and the five-parameter initiator-completer model ($p = 0.70$). All other submodels demonstrated significant lack of fit ($p < 0.0001$). The estimated parameters of the six-parameter model are given in Table 3, and the predicted probabilities of tumor are presented in Table 4, where the observed probabilities from Table 1 are reported for comparison purposes. As stated previously in "Two-Stage Clonal Expansion Model," neither the full initiator-promoter-completer model nor the initiator-completer model can be extrapolated from a continuous to a discontinued dosing situation due to a lack of identifiability of parameters. Thus the only model left for such extrapolation was the initiator-promoter model. That model gave an acceptable fit to the 16 continuous dosing data points ($p = 0.71$) and also fit well when extrapolated to the 24 discontinued dosing data points ($p = 0.68$). However, as previously noted, the continuous dosing version of the initiator-promoter model is indistinguishable from the promoter-completer model. And the promoter-completer model, as would be expected since it did not fit the 40 data points initially, demonstrated a significant lack of fit when extrapolated from the continuous to the discontinued dosing data ($p > 0.00001$).

It is not possible to choose which, if any, of the three models that fit acceptably to all 40 liver tumor data points is the "correct" model. That is, the present bioassay data do not contain the refined biological information necessary to choose one model over the others. In a sense, it is gratifying that the full two-stage model with colonal expansion, the initiator-promoter-completer model, fits the data. However, it is not very satisfying that two submodels, the initiator-promoter and initiator-completer models, are approximately equally acceptable, since they represent two different biological mechanisms. From a statistical point of view, the initiator-promoter model might be preferred, since it has the fewest parameters and since it was possible to demonstrate for that model successful extrapolation from the continuous to the discontinued dosing situation. The importance of discontinued dosing experiments is high-

Table 3. Estimated parameters of the six-parameter, two-stage clonal expansion model fitted to liver and bladder tumor data.

Carcinogenic end point	α_1	β_1	α_2	β_2	α_3	β_3
Liver tumor[a]	2.1×10^{-6}	2.1×10^{-7}	3.5×10^{-1}	1.8×10^{-3}	3.4×10^{-1}	1.9×10^{-5}
	(1.8×10^{-6})[b]	(9.0×10^{-8})	(0.0)[c]	(5.4×10^{-4})	(2.3×10^{-2})	(8.6×10^{-5})
Bladder tumor[d]	0.0	2.8×10^{-6}	1.4×10^{-1}	2.7×10^{-1}	1.9×10^{-9}	2.7×10^{-3}
	(1.8×10^{-3})	(1.9×10^{-4})	(9.2)	(1.7×10^{-2})	(9.0×10^{-3})	(6.0×10^{-4})

[a]Goodness-of-fit p-value = 0.76.
[b]Estimated SE in parentheses.
[c]Information matrix was singular.
[d]Goodness-of-fit p-value < 0.00001.

Table 4. Comparison of observed and predicted probabilities of liver tumor for the full, six-parameter, two-stage clonal expansion model.

	Rooms 141, 142, 143 combined[a]				Rooms 144, 145, 146 combined[a]			
D[b]	T_1[b]	T[b]	$\hat{P}$ (obs)[c]	$\hat{P}$ (pred)[c]	T_1	T	$\hat{P}$ (obs)	$\hat{P}$ (pred)
0	24	24	0.034	0.021	18	18	0.005	0.003
30	24	24	0.080	0.096	18	18	0.016	0.013
35	24	24	0.097	0.110	18	18	0.015	0.015
45	24	24	0.148	0.139	18	18	0.024	0.019
60	24	24	0.179	0.185	18	18	0.028	0.026
75	24	24	0.229	0.232	18	18	0.030	0.033
100	24	24	0.304	0.315	18	18	0.055	0.047
150	24	24	0.494	0.481	18	18	0.068	0.080
60	9	24	0.124	0.137	9	18	0.005	0.019
60	12	24	0.117	0.141	12	18	0.010	0.020
60	15	24	0.156	0.144	15	18	0.025	0.022
75	9	24	0.188	0.164	9	18	0.038	0.023
75	12	24	0.210	0.169	12	18	0.045	0.025
75	15	24	0.195	0.173	15	18	0.038	0.027
100	9	24	0.158	0.208	9	18	0.016	0.030
100	12	24	0.213	0.215	12	18	0.000	0.033
100	15	24	0.245	0.221	15	18	0.016	0.037
150	9	24	0.294	0.289	9	18	0.078	0.045
150	12	24	0.333	0.300	12	18	0.047	0.049
150	15	24	0.256	0.310	15	18	0.071	0.058

[a]A sacrifice at 18 months occurred only in rooms 144, 145, 146; a sacrifice at 24 months occurred only in rooms 141, 142, 143.
[b]D represents dose of 2-AAF in parts per million; T_1 represents time of termination of dosing expressed in months; T represents time of observation of time-to-tumor-onset distribution expressed in months.
[c]$\hat{P}$ (obs) is the nonparametric estimate of the probability of tumor onset from Table 1. $\hat{P}$ (pred) is the predicted probability of tumor onset from the two-stage colonal expansion model.

lighted by the rejection of the promoter-completer model based on the discontinued dosing data, whereas that model is indistinguishable with continuous dosing data from the very successful but mechanistically very different initiator-promoter model.

For the bladder tumor data (Table 2), none of the seven versions of the two-stage clonal expansion model fit acceptably ($p < 0.00001$), whether all 40 data points or just the 16 continuous dosing data points were used. Table 3 gives the estimated parameters for the full, six-parameter model fitted to the 40 data points in Table 2. The predicted probabilities of tumor for the six-parameter model are given in Table 5, along with the observed probabilities from Table 2.

It is quite disappointing that the two-stage clonal expansion model would not fit even the 16 continuous dosing data points. Several possible remedies were investigated in an attempt to achieve an acceptable fit of the model to these data. The pharmacokinetic dose transformation of Whittemore et al. (*19*), $d' = a_1 d^k (1 + a_2 d^k)$, was tried for k = 1,2, which resulted in the estimation of one additional parameter (a_2). This is the familiar Michaelis-Menten transformation when k = 1. A power transformation, $d' = d^k$, also was attempted for k = 2 to 4. This was used first throughout the full model and then only in the cell proliferation function. None of these remedies provided an appreciable improvement in the fit of the model.

It has been the experience of other investigators with other mechanistically derived models that the ED_{01} liver tumor data are consistent with a variety of such models (*5,6*), but that the ED_{01} bladder tumor data are not ad-

Table 5. Comparison of observed and predicted probabilities of bladder tumor for the full, six-parameter, two-stage clonal expansion model.

	Rooms 141, 142, 143 combined[a]				Rooms 144, 145, 146 combined[a]			
D[b]	T_1[b]	T[b]	$\hat{P}$ (obs)[c]	$\hat{P}$ (pred)[c]	T_1	T	$\hat{P}$ (obs)	$\hat{P}$ (pred)
0	24	24	0.007	0.000	18	18	0.002	0.000
30	24	24	0.008	0.007	18	18	0.003	0.003
35	24	24	0.004	0.010	18	18	0.002	0.004
45	24	24	0.012	0.018	18	18	0.006	0.007
60	24	24	0.009	0.041	18	18	0.010	0.014
75	24	24	0.023	0.091	18	18	0.004	0.026
100	24	24	0.173	0.316	18	18	0.051	0.071
150	24	24	0.802	0.999	18	18	0.531	0.457
60	9	24	0.020	0.009	9	18	0.000	0.006
60	12	24	0.020	0.014	12	18	0.000	0.009
60	15	24	0.000	0.020	15	18	0.005	0.012
75	9	24	0.013	0.013	9	18	0.016	0.009
75	12	24	0.015	0.023	12	18	0.000	0.015
75	15	24	0.019	0.037	15	18	0.008	0.021
100	9	24	0.000	0.026	9	18	0.016	0.018
100	12	24	0.047	0.052	12	18	0.030	0.033
100	15	24	0.043	0.096	15	18	0.000	0.053
150	9	24	0.188	0.087	9	18	0.063	0.059
150	12	24	0.346	0.229	12	18	0.234	0.145
150	15	24	0.429	0.503	15	18	0.368	0.299

[a]A sacrifice at 18 months occurred only in rooms 144, 145, 146; a sacrifice at 24 months occurred only in rooms 141, 142, 143.

[b]D represents dose of 2-AAF in parts per million; T_1 represents time of termination of dosing expressed in months; T represents time of observation of time-to-tumor-onset distribution expressed in months.

[c]$\hat{P}$ (obs) is the nonparametric estimate of the probability of tumor onset from Table 2. $\hat{P}$ (pred) is the predicted probability of tumor onset from the two-stage clonal expansion model.

equately described by any of them (*6,7*). It appears that the two-stage clonal expansion model as formulated in this paper may be characterized the same way. For this reason, a different approach is proposed in the next section.

Weibull-Type Model for Discontinued Dosing

The model to be proposed here is more empirically based than biologically based. Although the Weibull distribution can arise naturally in the theory of systems reliability, its application to biological problems has consisted mostly of empirical curve fitting. In the analysis of toxicity data, both dose and time have been modeled separately as Weibull random variables. When dose and time have appeared together in Weibull-type models, generally time has been considered the random variable (*20*). The commonly used continuous dosing version of the multistage model of Armitage and Doll (*4*) may be considered a Weibull model in time, with the polynomial function in dose representing a scale parameter.

Peto et al. (*20*) used two versions of a Weibull model to describe carcinogenesis data on nitrosamines in a study with a large number of rodents. In this discussion, these two models will be distinguished according to whether background is modeled as "additive" or "independent." The cumulative hazard for the additive background model is

$$H(d,t) = (\alpha+\beta d)^w t^k,$$

and that for the independent background model is

$$H(d,t) = (\alpha+\beta d^w) t^k,$$

where α, β, w, and k are all nonnegative constants. For liver tumors that occurred under a continuous dosing regimen, Peto et al. (*20*) found that the additive background model described the data best, there being an appreciable background incidence of liver tumors. For esophageal tumors observed under continuous dosing, Peto et al. (*20*) found that the independent background model fit best; in fact, the observed background incidence of esophageal tumors was zero, so that α was zero in the model for esophageal tumors.

The parallels between the nitrosamine-induced liver tumors and the ED_{01} study's 2-AAF induced liver tumors and between the nitrosamine-induced esophageal tumors and the ED_{01} study's bladder tumors suggest that a Weibull model with additive background might fit the ED_{01} liver tumor data and that a Weibull model with independent background might fit the ED_{01} bladder tumor data. But how does one generalize the Weibull model to the discontinued dosing situation? Unlike mechanistically derived models, the Weibull model does not have a natural extension to discontinued dosing.

One generalization of the Weibull model to discontinued dosing was given by Carlborg (*21*). He modeled the ED_{01} tumor prevalence data using only what is described above as the independent background model. His generalization to discontinued dosing included a component for duration of dosing, but did not account for when the dosing occurred. Carlborg (*21*) successfully fit a five-parameter model to the ED_{01} liver tumor prevalence data, excluding a portion of the low-response continuous dosing data, but including all the discontinued dosing data. However, Carlborg (*21*) did not fit the bladder tumor data on dose and time simultaneously. Apparently, the model would not fit.

Freedman and Navidi (*6*) looked briefly at fitting the Weibull model to ED_{01} bladder tumor prevalence data. They were unable to obtain an acceptable fit, but noted a tendency for the dose exponent, w, to exceed the time exponent, k, which they noted to be inconsistent with the multistage model. Freedman and Navidi (6) quoted the argument of Brown and Hoel (*22*) that a factorable hazard model (like the Weibull or multistage) will not fit the continuous dosing bladder tumor data over all dose and time points. Brown and Hoel (*22*) made a similar but weaker statement about the liver tumor data, which Carlborg (*21*) was able to fit acceptably, along with the discontinued dosing data, using a factorable Weibull model. The model that will now be proposed is a discontinued dosing extension of the Weibull model used by Peto et al. (*20*).

First consider additive background. Assume initially that dosing occurs only between time t_0 and t_1 and at a constant rate, d. The model can easily be generalized to multiple periods of constant exposure, as will be discussed later. Let τ represent a latency parameter, that is, a minimum amount of time that must elapse before a tumor can be observed. Suppose that $t_0 \le \tau < t_1$. Then

$$H(d,t) = 0 , \qquad t \le \tau ,$$

and

$$H(d,t) = (\alpha+\beta_1 d)^w (t-\tau)^k , \quad \tau < t \le t_1 ,$$

where β has been replaced by β_1 for notational purposes. If administered dose is abruptly discontinued, there is no reason to assume that the effective dose will immediately vanish. To generalize to time $t > t_1$, suppose that the general form of H(d,t) is the same but that time to tumor is increased by a location shift of the distribution to the right and that the dose is effectively reduced by a scale factor. That is, let

$$H(d,t) = (\alpha+\beta_2 d)^w (t-\tau-c)^k , \quad t_1 \le t ,$$

where $c > 0$ and $\beta_2 < \beta_1$. In order for this generalization to be valid, at time t_1, the condition

$$(\alpha+\beta_1 d)^w (t_1-\tau)^k = (\alpha+\beta_2 d)^w (t_1-\tau-c)^k$$

must be satisfied. This implies that c must be equal to $(t_1-\tau)[1 - \{(\alpha+\beta_1 d)/(\alpha + \beta_2 d)\}^{w/k}]$, which in turn implies that

$$H(d,t) = [(\alpha+\beta_2 d)^{w/k} (t-t_1) + (\alpha+\beta_1 d)^{w/k} (t_1-\tau)]^k , \quad t_1 \le t .$$

This generalized Weibull model (with $t_0 \le \tau < t_1$) can be applied to the ED_{01} liver and bladder tumor data. The independent background version is derived similarly and may be expressed as

$$H(d,t) = \begin{cases} 0 , & t \le \tau , \\ (\alpha+\beta_1 d^w)(t-\tau)^k , & \tau < t \le t_1 , \\ [(\alpha+\beta_2 d^w)^{1/k}(t-t_1) + (\alpha+\beta_1 d^w)^{1/k}(t_1-\tau)]^k , & t_1 \le t . \end{cases}$$

Other versions of this generalized Weibull model depend on the relative magnitude of τ with respect to t_0 and t_1. For $0 \le \tau < t_0$, the additive background model has

$$H(d,t) = [\alpha^{w/k}(t_0-\tau) + (\alpha+\beta_1 d)^{w/k}(t_1-t_0) + (\alpha+\beta_2 d)^{w/k}(t-t_1)]^k , \quad t_1 \le t ,$$

with an analogous expression for the independent background model. If $t_1 \le \tau$, then the additive background model's cumulative hazard is

$$H(d,t) = [(\alpha+\beta_2 d)^{w/k}(t-\tau)]^k = (\alpha+\beta_2 d)^w (t-\tau)^k , \quad t_1 \le t ,$$

with an analogous expression for the independent background model. With regard to multiple periods of constant exposure, the cumulative hazard can easily be derived in the same way as for a single exposure period. It will have a similar appearance, but with additional terms inside the bracketed expression raised to the k*th* power.

Results for Weibull Discontinued Dosing Model

For the liver tumor data (Table 1), the additive background model fit acceptably to the 40 data points ($p = 0.43$), as did the independent background model ($p = 0.63$). The estimated value of τ was essentially zero (7.2×10^{-17}) for the additive model, whereas the estimated value was 0.81 for the independent model. Parameter estimates for the additive model, for which the prediction of background tumor rates was slightly better, are given in Table 6. The estimated power on dose ($w = 1.7$) and the estimated power on time ($k = 7.7$) are reasonably close to values (2 and 7, respectively) found by Freedman and Navidi (*6*) for the dose and time powers in the multistage model. Table 7 compares the predicted probabilities of the six-parameter additive background model to the observed probabilities from Table 1.

Table 6. Estimated parameters of Weibull discontinued dosing models fitted to liver and bladder tumor data.

Carcinogenic end point	Modeling of background	α	β_1	β_2	τ	w	k
Liver tumor[a]	Additive	8.8×10^{-8}	2.8×10^{-9}	1.1×10^{-9}	7.2×10^{-17}	1.7	1.7
		(8.4×10^{-7})[b]	(2.4×10^{-8})	(9.6×10^{-9})	(1.3×10^{-16})	(1.3)	(1.3)
Bladder tumor[c]	Independent	1.1×10^{-5}	1.8×10^{-16}	9.1×10^{-18}	7.0	6.1	2.2
		(2.8×10^{-4})	(1.5×10^{-14})	(1.2×10^{-15})	(8.8)	(0.7)	(2.7)

[a]Goodness-of-fit p-value = 0.43.
[b]Estimated SE in parentheses.
[c]Goodness-of-fit p-value = 0.47.

For the bladder tumor data (Table 2), the independent background model fit acceptably to the 40 data points ($p = 0.47$), whereas the additive background model did not ($p < 10^{-7}$). Practically all the lack of fit of the additive background model appeared to be in the background itself. Parameter estimates for the independent model are given in Table 6. The relative magnitudes of the estimated power on dose (w = 6.1) and the estimated power on time (k = 2.2) are inconsistent with a multistage formulation in administered dose (wherein the power on time must equal or exceed the power on dose), a phenomenon noted by Freedman and Navidi (*6*) in reference to Carlborg (*21*). The same is true for the two-stage model with clonal expansion; however, in both cases, a judicious transformation of dose might improve the fit. Table 8 compares the predicted probabilities of the six-parameter independent background model to the observed probabilities from Table 2.

The results in Tables 6, 7, and 8 indicate good fits and predictive ability of the given Weibull models within the range of observed doses (D), stopping times (T_1), and observation times (T). In order to evaluate the predictive ability of the models at points outside the range of data used for fitting, observation times T = 15 and 33 for continuous dosing were selected for prediction. The results are given in Table 9. The 15-month predictions appear consistent with the observed data, which is itself characterized by considerable variability about the lower dose rates. However, at 33 months the predictions appear to break down. The liver tumor model is characterized by overprediction at the middle dose levels, while the bladder tumor model is characterized by substantial underprediction at 75 and 100 ppm.

Table 7. Comparison of observed and predicted probabilities of liver tumor for the six-parameter Weibull model with additive background.

	Rooms 141, 142, 143 combined[a]				Rooms 144, 145, 146 combined[a]			
D[b]	T_1[b]	T[b]	$\hat{P}$ (obs)[c]	$\hat{P}$ (pred)[c]	T_1	T	$\hat{P}$ (obs)	$\hat{P}$ (pred)
0	24	24	0.034	0.031	18	18	0.005	0.003
30	24	24	0.080	0.095	18	18	0.016	0.011
35	24	24	0.097	0.108	18	18	0.015	0.012
45	24	24	0.148	0.135	18	18	0.024	0.016
60	24	24	0.179	0.180	18	18	0.028	0.021
75	24	24	0.229	0.227	18	18	0.030	0.027
100	24	24	0.304	0.310	18	18	0.055	0.040
150	24	24	0.494	0.477	18	18	0.068	0.068
60	9	24	0.124	0.110	9	18	0.005	0.014
60	12	24	0.117	0.122	12	18	0.010	0.016
60	15	24	0.156	0.135	15	18	0.025	0.019
75	9	24	0.188	0.135	9	18	0.038	0.018
75	12	24	0.210	0.150	12	18	0.045	0.020
75	15	24	0.195	0.167	15	18	0.038	0.024
100	9	24	0.158	0.178	9	18	0.016	0.024
100	12	24	0.213	0.200	12	18	0.000	0.028
100	15	24	0.245	0.225	15	18	0.016	0.034
150	9	24	0.294	0.271	9	18	0.078	0.039
150	12	24	0.333	0.307	12	18	0.047	0.047
150	15	24	0.256	0.346	15	18	0.078	0.057

[a]A sacrifice at 18 months occurred only in rooms 144, 145, 146; a sacrifice at 24 months occurred only in rooms 141, 142, 143.
[b]D represents dose of 2-AAF in parts per million; T_1 represents time of termination of dosing expressed in months; T represents time of observation of time-to-tumor onset distribution expressed in months.
[c]$\hat{P}$ (obs) is the nonparametric estimate of the probability of tumor onset from Table 1. $\hat{P}$ (pred) is the predicted probability of tumor onset from the Weibull discontinued dosing model.

Table 8. Comparison of observed and predicted probabilities of bladder tumor for the six-parameter Weibull model with independent background.

	Rooms 141, 142, 143 combined[a]				Rooms 144, 145, 146 combined[a]			
D[b]	T_1[b]	T[b]	$\hat{P}$ (obs)[c]	$\hat{P}$ (pred)[c]	T_1	T	$\hat{P}$ (obs)	$\hat{P}$ (pred)
0	24	24	0.007	0.005	18	18	0.002	0.002
30	24	24	0.008	0.005	18	18	0.003	0.002
35	24	24	0.004	0.005	18	18	0.002	0.002
45	24	24	0.012	0.006	18	18	0.006	0.002
60	24	24	0.009	0.012	18	18	0.010	0.005
75	24	24	0.023	0.030	18	18	0.004	0.012
100	24	24	0.173	0.140	18	18	0.051	0.057
150	24	24	0.802	0.825	18	18	0.531	0.491
60	9	24	0.020	0.006	9	18	0.000	0.002
60	12	24	0.020	0.007	12	18	0.000	0.003
60	15	24	0.000	0.008	15	18	0.005	0.004
75	9	24	0.013	0.008	9	18	0.016	0.004
75	12	24	0.015	0.012	12	18	0.000	0.006
75	15	24	0.019	0.015	15	18	0.008	0.008
100	9	24	0.000	0.020	9	18	0.016	0.010
100	12	24	0.047	0.036	12	18	0.030	0.021
100	15	24	0.043	0.056	15	18	0.000	0.037
150	9	24	0.188	0.162	9	18	0.063	0.086
150	12	24	0.346	0.296	12	18	0.234	0.198
150	15	24	0.429	0.447	15	18	0.368	0.339

[a]A sacrifice at 18 months occurred only in rooms 144, 145, 146; a sacrifice at 24 months occurred only in rooms 141, 142, 143.

[b]D represents dose of 2-AAF in parts per million; T_1 represents time of termination of dosing expressed in months; T represents time of observation of time-to-tumor-onset distribution expressed in months.

[c]$\hat{P}$ (obs) is the nonparametric estimate of the probability of tumor onset from Table 2. $\hat{P}$ (pred) is the predicted probability of tumor onset from the Weibull discontinued dosing model.

Table 9. Prediction of tumor probabilities at 15 and 33 months based on models fitted to 18- and 24-month observations.

		Tumor type					
		Liver			Bladder		
D[a]	T[a]	$\hat{P}_1$(obs)[b]	$\hat{P}_2$(obs)[b]	$\hat{P}$ (pred)[b]	$\hat{P}_1$(obs)	$\hat{P}_1$(obs)	$\hat{P}$ (pred)
0	15	0.000	0.001	0.001	0.007	0.001	0.001
30	15	0.000	0.009	0.003	0.005	0.000	0.001
35	15	0.010	0.000	0.003	0.004	0.000	0.001
45	15	0.000	0.010	0.004	0.001	0.005	0.001
60	15	0.007	0.000	0.005	0.000	0.000	0.002
75	15	0.000	0.000	0.007	0.014	0.000	0.006
100	15	0.023	0.012	0.010	0.000	0.003	0.029
150	15	0.016	0.007	0.017	0.263	0.313	0.287
0	33	0.322	0.507	0.303	0.007	0.036	0.013
30	33	0.653	0.627	0.686	0.040	0.020	0.013
35	33	0.551	0.681	0.735	0.004	0.006	0.014
45	33	0.659	0.691	0.816	0.086	0.050	0.016
60	33	0.663	0.825	0.900	0.009	0.111	0.029
75	33	1.000	0.748	0.950	0.512	0.333	0.074
100	33	1.000	0.867	0.987	1.000	1.000	0.316
150	33	1.000	0.879	0.999	1.000	1.000	0.988

[a]D represents dose of 2-AAF in parts per million; T represents time of observation of time-to-tumor-onset distribution expressed in months.

[b]$\hat{P}_1$(obs) is the nonparametric estimate of the probability of tumor onset by time T based on data from rooms 141, 142, 143 combined; $\hat{P}_2$(obs) is the corresponding estimate from rooms 144, 145, 146 combined; $\hat{P}$ (pred) is the predicted probability of tumor onset from the Weibull models described in Table 6.

Discussion

It is disappointing that the two-stage model with proliferation of initiated cells would not fit the bladder tumor data from the ED_{01} study. The experience here, like that of previous investigators (*6,7,22*), indicates that the relationship between dose of 2-AAF and time to bladder tumor might be too complex to be explained by mechanistic models so far postulated. In the present case, even allowing for the Michaelis-Menten and other transformations of the administered dose, an acceptable fit was not obtained.

The fact that the two-stage clonal expansion model did fit the ED_{01} study's liver tumor data is interesting

and helps to narrow down the possible characterizations of liver carcinogenesis within the context of the two-stage clonal expansion model. Although a complete characterization was not possible, at least the simplest notions of pure initiator, pure promoter, and pure completer could be eliminated. The importance of discontinued dosing experiments is highlighted by the fact that the promoter-completer model was rejected, while the initiator-promoter model was not, the two models being indistinguishable for continuous data only. It is probably not advisable to make too much of the two-stage clonal expansion model's fit to the liver tumor data. For one thing, various other models have provided equally acceptable fits. For another, the model's failure to fit the bladder tumor data makes its biological interpretation less compelling.

With respect to the Weibull-type model that did fit acceptably to the 18- and 24-month data on both liver and bladder tumors, what conclusions can be drawn? Unfortunately, this model too broke down when extrapolated to tumor probabilities at 33 months. However, the good fits to the discontinued dosing data and the successful extrapolation to 15 months of continuous dosing give confidence that good predictions can be made for both liver and bladder tumor probabilities up to 24 months, regardless of when dosing stops. The importance of discontinued dosing experiments is again emphasized. Whether a model is mechanistically based or simply empirically based, its ability or inability to explain discontinued dosing tumor data will provide a good measure of its predictive capability beyond the experimental setting.

In addition to the discontinued dosing version of the Weibull-type model presented here, there are other formulations that might be useful. One such model that was investigated in this study but not reported had only four parameters. Instead of the additional scale parameter β_2 being introduced, the original parameter β_1 was retained, but the dose was transformed to the time-weighted-average dose. This model gave an acceptable fit for both liver and bladder tumors. However, the bladder tumor fit was not as good as that of the model reported here. The advantage of such a model would be its extrapolation potential to discontinued dosing situations based on parameters estimated from only continuous dosing data.

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Appendix

Derivation of Hazard Function of Two-Stage Clonal Expansion Model for Single, Less-Than-Lifetime Period of Dosing

Assume that dosing occurs at a constant rate d between time t_0 and t_1, and is zero otherwise, and that the experiment continues until time t, where $0 \le t_0 < t_1 < t$. Then the instantaneous hazard is

$$h(d,w;t_0,t_1) = \alpha_2 \int_0^w N\,\alpha_1 \exp\{\int_v^w \alpha_3\,du\}\,dv$$

$$= N\frac{\alpha_1\alpha_2}{\alpha_3}[\exp(\alpha_3 w)-1], \qquad w \le t_0.$$

$$h(d,w;t_0,t_1) = (\alpha_2+\beta_2 d)\int_0^{t_0} N\alpha_1 \exp\{\int_v^{t_0}\alpha_3\,du + \int_{t_0}^{w}(\alpha_3+\beta_3 d)\,du\}\,dv + (\alpha_2+\beta_2 d)\int_{t_0}^{w} N(\alpha_1+\beta_1 d)\exp\{\int_v^w(\alpha_3+\beta_3 d)\,du\}\,dv$$

$$= N\frac{\alpha_1(\alpha_2+\beta_2 d)}{\alpha_3}\exp\{(\alpha_3+\beta_3 d)(w-t_0)\}\,[\exp(\alpha_3 t_0)-1] + N\frac{(\alpha_1+\beta_1 d)(\alpha_2+\beta_2 d)}{\alpha_3+\beta_3 d}[\exp\{(\alpha_3+\beta_3 d)(w-t_0)\}-1], \quad t_0 \le w \le t_1.$$

$$h(d,w;t_0,t_1) = \alpha_2\int_0^{t_0} N\alpha_1\exp\{\int_v^{t_0}\alpha_3\,du + \int_{t_0}^{t_1}(\alpha_3+\beta_3 d)\,du + \int_{t_1}^{w}\alpha_3\,du\}\,dv + \alpha_2\int_{t_0}^{t_1} N(\alpha_1+\beta_1 d)\exp\{\int_v^{t_1}(\alpha_3+\beta_3 d)\,du + \int_{t_1}^{w}\alpha_3\,du\}\,dv + \alpha_2\int_{t_1}^{w} N\alpha_1\exp\{\int_v^{w}\alpha_3\,du\}\,dv$$

$$= N\frac{\alpha_1\alpha_2}{\alpha_3}\exp\{(\alpha_3+\beta_3 d)(t_1-t_0)+\alpha_3(w-t_1)\}\,[\exp(\alpha_3 t_0)-1] + N\frac{(\alpha_1+\beta_1 d)\alpha_2}{\alpha_3+\beta_3 d}\exp\{\alpha_3(w-t_1)\}\,[\exp\{(\alpha_3+\beta_3 d)(t_1-t_0)\}-1] + N\frac{\alpha_1\alpha_2}{\alpha_3}[\exp\{\alpha_3(w-t_1)\}-1], \quad t_1 \le w.$$

Of primary interest in this paper is the cumulative hazard function for $t_1 < t$. This is found by summing together the integrals of the above three instantaneous hazards, where the limits of integration on w are, respectively, 0 to t_0, t_0 to t_1, and t_1 to t. The resulting expression is

$$H(d,t;t_0,t_1) = N\frac{\alpha_1\alpha_2}{\alpha_3^2}[\exp(\alpha_3 t_0)-1-\alpha_3 t_0] + N\frac{\alpha_2+\beta_2 d}{\alpha_3+\beta_3 d}\left[\frac{\alpha_1}{\alpha_3}[\exp(\alpha_3 t_0)-1]\right.$$

$$[\exp\{(\alpha_3+\beta_3 d)(t_1-t_0)\}-1]$$

$$+\ \frac{\alpha_1+\beta_1 d}{\alpha_3+\beta_3 d}\,[\exp\{(\alpha_3+\beta_3 d)(t_1-t_0)\}-1-(\alpha_3+\beta_3 d)$$

$$(t_1-t_0)]\Bigg]+N\frac{\alpha_2}{\alpha_3}\left[\frac{\alpha_1}{\alpha_3}\exp\{(\alpha_3+\beta_3 d)(t_1-t_0)\}\right.$$

$$[\exp(\alpha_3 t_0)-1][\exp\{\alpha_3(t-t_1)\}-1]$$

$$+\ \frac{\alpha_1+\beta_1 d}{\alpha_3+\beta_3 d}\,[\exp\{(\alpha_3+\beta_3 d)(t_1-t_0)\}-1]$$

$$[\exp\{\alpha_3(t-t_1)\}-1]$$

$$+\ \left.\frac{\alpha_1}{\alpha_3}[\exp\{\alpha_3(t-t_1)\}-1-\alpha_3(t-t_1)\right],\quad t_1\le t.$$

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